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Host response of *Oryza glaberrima* and *O. sativa* rice genotypes to the rice root-knot nematode *Meloidogyne graminicola* in a hydroponic system under growth chamber

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The host response of 25 rice genotypes belonging to *Oryza glaberrima and Oryza sativa* to *Meloidogyne graminicola* infection was examined in a hydroponic system. The *M. graminicola* can build up high population densities in a hydroponic system. Resistance to this nematode species was found in *O. glaberrima* genotypes which supported significantly lower nematode numbers per plant and per unit root than *O. sativa* genotypes. The *M. graminicola*-infected *O. sativa* genotypes showed a higher root galling index than the *O. glaberrima* genotypes. The hydroponic system is efficient and reliable method to examine the host response of rice genotypes to *M. graminicola* infection, and can be useful for the fast screening of high numbers of rice genotypes for the selection of *M. graminicola*-resistant rice germplasm for breeding purposes.

Keywords: resistance; rice; root galling; screening; susceptibility

Introduction

The options to control *Meloidogyne graminicola* are limited. Studies on host plant resistance to plant-parasitic nematodes have increased in importance since the use of nematicides has been either banned or restricted in the recent past (Starr et al. 2002). In plant nematology, the host response of an agricultural crop to nematode infection is assessed by the ability of the crop to allow or suppress nematode reproduction and to suffer less or more damage. Plants (species or genotypes) can be either susceptible or resistant and sensitive or tolerant to nematode infection (Bos & Parlevliet 1995).

Following the terminology of Bos and Parlevliet (1995), resistance/susceptibility on the one hand and tolerance/sensitivity on the other hand are defined as independent, relative qualities of a host plant based on comparison between genotypes. A host plant may either suppress (resistance) or allow (susceptibility) nematode development and reproduction; it may suffer either little injury (tolerance), even when heavily infected with nematodes, or much injury (sensitivity), even when relatively lightly infected with nematodes. Resistance/susceptibility can be determined by assessing the nematode population densities in and on the roots, whereas tolerance/sensitivity can be determined

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by measuring the effect of the nematode population on plant growth, yield-contributing plant traits and/or yield (Cook & Evans 1987).

Relatively few studies have been published on the host response of rice germplasm to *M. graminicola* infection. Most of these studies were conducted either in pots filled with soil in a glasshouse, in outdoor raised beds filled with soil or under field conditions (Plowright et al. 1999; Soriano et al. 1999; Pokharel et al. 2011; De Waele et al. 2013; Win et al. 2013). All these methods allow the evaluation of both susceptibility/resistance to *M. graminicola* reproduction and sensitivity/tolerance to damage caused by *M. graminicola,* but all these methods also require a lot of space and time, and high inputs labour-wise and money-wise. For instance, the soil must be sterilised (sometimes large amounts of sterilised soil are necessary), usually a large number of nematodes is needed as primary inoculum, the plants are regularly irrigated, fertilised and protected against diseases, pests, etc. In addition, the environmental conditions may change during the season and among seasons, and efficient climatisation of the facilities (such as in a glasshouse) may not always be present due to its high cost and other factors. Field studies of the host response of rice genotypes to nematodes are also complicated by the occurrence of mixed populations of nematode species in the field (Villanueva et al. 1992) and by the uneven distribution of the nematode populations over the field (Hussey & Janssen 2002; Starr & Mercer 2009).

Up to now, sources of natural resistance to *M. graminicola* infection in the *Oryza* germplasm in general and in Asian rice (*Oryza sativa*) in particular are limited. Resistance has been found in *Oryza longistaminata* and in African rice (*Oryza glaberrima*, Plowright et al. 1999; Soriano et al. 1999; Cabasan et al. 2012) but attempts to introgress the resistance genes of *O. glaberrima* into *O. sativa* genotypes have failed so far as the interspecific progenies of the crosses did not express the same degree of resistance as observed in *O. glaberrima* (Plowright et al. 1999; De Waele pers. communication). With the continued efforts to find additional sources of natural resistance to *M. graminicola* in *Oryza* germplasm, and especially in *O. sativa*, a more practical screening method is needed which is faster, needs less space and inputs compared with the screening methods used so far.

About 20 years ago, Lambert et al. (1992) developed a hydroponic system for the culturing of the root-knot nematode *Meloidogyne javanica* on tomato plants grown in a nutrient solution. Subsequently, some hydroponic systems were developed for the culturing and study of *Meloidogyne* species (Synder et al. 2006 for the production of males of *M. incognita*; Oka & Mizukubo 2009 for the effect of culture filtrates on the hatching and activity of *M. incognita* J2) and for the synchronisation of similar developmental stages of *Meloidogyne* species, especially for molecular biology studies (Ji et al. 2013). In 1999, Reversat et al. developed a hydroponic system for the xenic culturing of a large variety of tropical nematode species, including *M. graminicola*, based on the use of a water-absorbent synthetic polymer (SAP: sand + absorbent polymer) as substrate.

In this study, we have made use of the hydroponic system developed by Reversat et al. (1999) as a more practical screening method to evaluate the host response of *O. sativa* genotypes to *M. graminicola* infection.

Materials and methods

Nematode inoculum

A *M. graminicola* population originally isolated from naturally infected Asian rice (genotype name unknown) in Batangas, Philippines, was cultured in a glasshouse under

upland conditions on the susceptible upland Asian rice genotype UPLRi-5. The inoculum was prepared by extraction of J2 from galled roots using the mistifier technique (Seinhorst 1950). Galled roots were carefully washed, cut into 5–10-mm pieces and incubated in a mist chamber. The *M. graminicola* J2 extracted after 48 h of incubation were used as inoculum.

Rice genotypes

The characteristics of the 25 rice genotypes examined (5 *O. glaberrima* and 20 *O. sativa* genotypes) in the hydroponic host response system are presented in Table 1. The genotypes UPLRi-5 and TOG5675 (an *O. glaberrima* genotype originating from Nigeria in West Africa) were included in the experiment as the susceptible and resistant reference genotype, respectively (Soriano et al. 1999).

Nutrient solution

Hoagland's nutrient solution provided the mineral requirements for plant growth in the hydroponic system. The following stock solutions were prepared: 2.5 M potassium nitrate, 0.5 M potassium dihydrogenophosphate, 2.5 M calcium nitrate, 1 M magnesium sulphate, micronutrients (H_3BO_3, $MgSO_4 \cdot 7H_2O$, $ZnSO_4 \cdot 7H_2O$, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, $CuSO_4 \cdot 5H_2O$, distilled water) and Fe-EDDHA (Reversat et al. 1999). Hoagland's

Table 1. Characteristics of the *O. sativa* and *O. glaberrima* genotypes included in the hydroponic host response experiment.

Genotype	Parentage	Ecotype	Species
Apo	UPLRi-5/IR12979-24-1	Aerobic	*O. sativa*
Aus196	Aus196	Upland	*O. sativa*
Aus257	Aus257	Upland	*O. sativa*
B6144F-MR-6-0-0	IRAT112/IR50	Aerobic	*O. sativa*
CT6510-24-1-2	P 5618/COL1XM312A-74-2-8-8	Aerobic	*O. sativa*
IR60080-46A	IR47686-8-4-3/CT6516-21-4-4	Aerobic	*O. sativa*
IR71525-19-1-1	IR60080-46A/IR62752-7	Aerobic	*O. sativa*
IR72	R19661-9-2-3/IR15795-199-3-3// IR9129-209-2-2-2-1	Lowland	*O. sativa*
IR78877-163-B-1-1	APO/IR72	Aerobic	*O. sativa*
IR78877-208-B-1-2	APO/IR72	Aerobic	*O. sativa*
IR78878-53-2-2-2	APO/IR72875-94-3-3-2	Aerobic	*O. sativa*
IR78910-23-1-3-4	Vandana/IR72	Aerobic	*O. sativa*
IR80508-B-194-3-B	APO/Aus257	Aerobic	*O. sativa*
Palawan	Palawan	Upland	*O. sativa*
UPLRi-5^{Susceptible}	Sigadis/BPI 76-1	Upland	*O. sativa*
UPLRi-7	C22/IR26/C22/OS 4	Aerobic	*O. sativa*
Vandana	C22/KALAKERI	Upland	*O. sativa*
WAB 450-24-2-3-P-38-1-HB	WAB 56-104/CG14	Aerobic	*O. sativa*
WAB 880 SG 42	WAB 56-50/CG14	Aerobic	*O. sativa*
Way Rarem	IR9669/B981	Aerobic	*O. sativa*
CG14	CG14	Aerobic	*O. glaberrima*
TOG5675^{Resistant}	TOG5675	Lowland	*O. glaberrima*
TOG5674	TOG5674	Lowland	*O. glaberrima*
TOG5681	TOG5681	Lowland	*O. glaberrima*
TOG7235	TOG7235	Lowland	*O. glaberrima*

nutrient solution (1/1) was prepared by adding 2 ml of each stock solution to 1000 ml deionised water. The Hoagland nutrient solution (1/1) was diluted with deionised water to 1/4 of its strength for the hydroponic system used in the experiment.

Hydroponic apparatus

The hydroponic apparatus was made up of small 30-mm diameter transparent polysterene (PS) tubes with a 70-mm long bottom tube and a 100-mm long cover tube, on one side open, the other side closed (Figure 1). The open ends of the tubes were connected together with adhesive tape. The bottom tube (that contained the SAP substrate, a mixture of pure silica sand and water-absorbent synthetic polymer) has a 5-mm diameter hole on the side for nematode inoculation and watering of the plant, and a 1-mm diameter hole at the base which provided a small drainage opening to maintain the SAP substrate at field capacity (i.e. 50% of SAP pore volume filled with water). The cover tube has two 5-mm diameter holes at the side which provided aeration for the plant and one 5-mm diameter hole at the top for watering of the plants (Reversat & Fernandez 2004). After 1 week of growing the plants in SAP substrate, the plants were transferred in the same type of tubes (but without a drainage hole) containing the nutrient solution (Figure 2).

Experimental set-up

The bottom tubes were filled with 15 g of the SAP substrate, moistened with water and one 2-day-old pre-germinated rice seedling was planted per tube. The bottom tube was connected to the cover tube and placed in an indoor growth chamber (IGC) at 29/26 °C (day/night temperature) with 12-h photoperiod. There were eight replicates per genotype. The experimental design was a randomised complete block design (RCB).

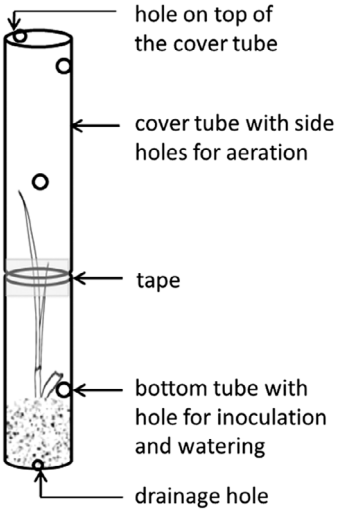

Figure 1. Hydroponic apparatus made up of a transparent PS tube for growing rice plants inoculated with *M. graminicola* in a sand + absorbent polymer (SAP) substrate.

Figure 2. Hydroponic apparatus and experimental set-up of the hydroponic system to evaluate the host response of rice genotypes to *M. graminicola* infection: (a) plant in sand + absorbent polymer (SAP) substrate, (b) plant in nutrient solution and (c) experimental set-up in the IGC.

Five days after transplanting, the seedlings were inoculated with 50 J2 per tube. Hoagland's nutrient solution was added twice a week and plants were watered at field capacity. One week after nematode inoculation, the cover tubes were removed and the seedlings were carefully uprooted from the SAP substrate. The roots were washed with distilled water to remove the adhering SAP particles. After washing, the seedlings were transferred into clean PS tubes without SAP substrate but with 15 ml of Hoagland's nutrient solution to maintain the plants under hydroponic conditions for 16 days. Thirty days after seed germination, i.e. 23 days after nematode inoculation which is about equivalent to a single life cycle of the *M. graminicola* Batangas population (Fernandez et al. 2013), the top and bottom tubes were separated and the nutrient solution was examined for the presence of J2 that had moved out of the roots. The J2 were counted using a stereomicroscope. The plants were removed from the tubes and the severity of root galling was evaluated following a rating scale of 0–5, wherein 0 = no galls, 1 = < 10% of the root system galled, 2 = 10% to < 25% of the root system galled, 3 = 25% to < 50% of the root system galled, 4 = 50% to < 75% of the root system galled and 5 = > 75% of the root system galled (Hussey & Janssen 2002).

After the fresh root weight was determined, the roots were cut into 1-cm pieces and incubated for 14 days in a mistifier for nematode (J2) extraction (Seinhorst 1950). After 14 days in the mistifier, the nematodes were collected and the number of J2 counted using a stereomicroscope. The nematode multiplication factor (Mf) was calculated as the final number of J2 counted in the nutrient solution + the number of J2 extracted from the roots/50 (50 J2 = the inoculated number of J2 per tube). The Mf of each rice genotype included in the experiment was compared with the Mf of the susceptible and resistant reference genotypes. Classification of the host response of the rice genotypes as resistant, partially resistant, susceptible or inconclusive was based on the methodology used for the first time by Dochez et al. (2005, Table 2).

Statistical analysis

Nematode counts were subjected to $\log(x + 1)$-transformation prior to analysis to meet the assumptions (i.e. homogeneity of variances and normality) of analysis of variance (ANOVA). Data were analysed using STATISTICA software (Statsoft Inc., Tulsa, USA).

Homogeneity of the variances of the groups was tested with the Levene test and Shapiro–Wilk test was used to examine whether the dependent variable was normally distributed within groups. One-way ANOVA was performed for comparisons between the means of the treatments. Dunnett's test ($p < 0.05$) was used to compare nematode reproduction on the rice genotypes with the reference genotypes UPLRi-5 (susceptible) and TOG5675 (resistant).

Results

When grown in a hydroponic system, the fresh root weights of the *O. sativa* and *O. glaberrima* genotypes infected with *M. graminicola* were, on average, comparable (0.27 and 0.31 g, respectively) at 30 days after germination.

Genotypes of *O. glaberrima* and *O. sativa* differed in their abilities to support *M. graminicola* reproduction. The number of J2 recovered per root unit (0.1 g roots) from the *O. sativa* genotypes was, on average, 800 times higher compared with the *O. glaberrima* genotypes (2549 vs. 3 J2). The nematode Mf in *O. sativa* genotypes was on average also 593 times higher compared with the *O. glaberrima* genotypes. In *O. sativa* genotypes, the *M. graminicola* population per root system increased 32–203 times in 3 weeks after inoculation with 50 J2 per plant.

The *O. glaberrima* reference genotype TOG5675 showed resistance to *M. graminicola* (Mf = 0), while the *O. sativa* reference genotype UPLRi-5 was susceptible to this nematode species (Mf = 161). A high Mf was observed for each *O. sativa* genotype compared with TOG5675. The aerobic genotype CT6510-24-1-2 had the lowest final nematode population among the *O. sativa* genotypes (Mf = 32), however, this value was still significantly ($p < 0.05$) higher compared with TOG5675. The aerobic genotype Way Rarem had the highest final nematode population among the *O. sativa* genotypes (Mf = 203). The Mf of all *O. sativa* genotypes was not significantly different from UPLRi-5 and significantly different from TOG5675. Therefore, all *O. sativa* genotypes included in the experiment are considered susceptible. On the other hand, *M. graminicola* did not reproduce on the *O. glaberrima* genotypes CG14, TOG5674, TOG5675, TOG5681 and TOG7235 which are all considered resistant (Table 3).

The *O. sativa* genotypes included in this study infected with *M. graminicola* showed a higher root galling index, on average 2.7, compared with the *O. glaberrima* genotypes which had a root galling index of, on average, 0.3. The root galling index of the *O. sativa* genotypes was not significantly different compared with the susceptible reference genotype UPLRi-5, except IR78910-23-1-3-4 which had the lowest root galling index (1.5) of the *O. sativa* genotypes. The *O. glaberrima* genotypes had comparable or

Table 2. Identification of the host response of *O. glaberrima* and *O. sativa* genotypes to *M. graminicola* infection based on a comparison with the response of a susceptible and a resistant reference rice genotype.

Comparison with the susceptible reference genotype	Comparison with the resistant reference genotype	Host response of the genotype tested
Significantly[a] different	Not significantly different	Resistant
Not significantly different	Significantly different	Susceptible
Significantly different	Significantly different	Partially resistant
Not significantly different	Not significantly different	Inconclusive

[a]According to Dunnett's test ($p < 0.05$).

Table 3. Host response of *O. sativa* and *O. glaberrima*^{Og} genotypes to *M. graminicola* infection grown in a hydroponic system at 30 days after germination and 23 days after inoculation with 50 second stage juveniles (J2) per plant.

Rice genotype	Fresh root weight (g)	No. of J2 per 0.1 g roots	No. of J2 per root system		Mf	Host response	Root galling index
Apo	0.4	4292	8004	ns[1],*[2]	160	S	2.1
Aus196	0.2	4340	6376	ns,*	128	S	2.3
Aus257	0.2	2116	4433	ns,*	89	S	2.9
B6144F-MR-6-0-0	0.2	2395	5268	ns,*	100	S	2.3
CT6510-24-1-2	0.2	933	1612	ns,*	32	S	2.1
IR60080-46A	0.3	1953	4871	ns,*	94	S	2.0
IR71525-19-1-1	0.3	3146	7988	ns,*	160	S	2.6
IR72	0.4	1083	3243	ns,*	65	S	2.5
IR78877-163-B-1-1	0.2	1275	2157	ns,*	43	S	2.5
IR78877-208-B-1-2	0.4	2962	6195	ns,*	124	S	3.6
IR78878-53-2-2-2	0.2	2431	4320	ns,*	86	S	2.3
IR78910-23-1-3-4	0.3	1644	2323	ns,*	46	S	1.5
IR80508-B-194-3-B	0.2	2317	5191	ns,*	104	S	3.8
Palawan	0.3	3798	9321	ns,*	186	S	2.9
UPLRi-5^{Susceptible}	0.4	2406	8578	–, *	161	–	4.1
UPLRi-7	0.3	1717	4426	ns,*	89	S	2.4
Vandana	0.3	2310	5914	ns,*	118	S	2.6
WAB 450-24-2-3-P-38-1-HB	0.3	1209	4073	ns,*	81	S	3.3
WAB 880 SG 42	0.2	1340	2997	ns,*	60	S	2.0
Way Rarem	0.2	7306	10,171	ns,*	203	S	3.5
CG14^{Og}	0.4	1	5	*, ns	0	R	0.9
TOG5674^{Og}	0.3	2	8	*, ns	0	R	0.0
TOG5675$^{Og\ Resistant}$	0.3	5	15	*, –	0	–	0.1
TOG5681^{Og}	0.2	1	1	*, ns	0	R	0.0
TOG7235^{Og}	0.4	6	17	*, ns	0	R	0.4

Notes: J2 in the root system + J2 in the nutrient solution (= final nematode population density); Mf: nematode multiplication factor = final nematode population density per root system/initial population density (= 50 J2); ns: not significantly different, *: significantly different from the susceptible genotype UPLRi-5([1]) or the resistant genotype TOG5675([2]) according to Dunnett's test ($p < 0.05$). Data were log($x + 1$)-transformed before analysis; –: reference genotype; host response: comparison between the number of J2 per root system of the genotype and the susceptible reference genotype UPLRi-5 or the resistant reference genotype TOG5675. S: susceptible, R: resistant to *M. graminicola* infection, root galling index on a scale from 0 to 5.

somewhat higher root galling indices (ranging from 0 to 0.9) compared with the resistant reference genotype TOG5675 (0.1).

Discussion

Under hydroponic conditions, the nematode-infected *O. glaberrima* and *O. sativa* genotypes did not show differences in fresh root weight. The same observation was made by De Waele et al. (2013) on mature plants of both rice species grown in a *M. graminicola*-infested sandy loam soil in outdoor raised beds under aerobic conditions.

Using the hydroponic system, it was possible to identify the *Oryza* genotypes (in this case all five *O. glaberrima* genotypes included in the experiment) which were resistant to *M. graminicola* infection. The same host response to *M. graminicola* infection of

these *O. glaberrima* genotypes was observed in many experiments conducted at IRRI (De Waele et al. 2013; De Waele pers. communication). This observation shows that the mechanism of resistance to *M. graminicola* was already active at this early plant growth stage and under hydroponic conditions. In the experiment reported by De Waele et al. (2013) the same *O. glaberrima* genotypes were grown under aerobic conditions in outdoor raised beds filled with a sandy loam soil until maturity and harvest.

None of the *O. sativa* genotypes included in the hydroponic host response experiment were resistant to *M. graminicola* infection. This is in agreement with the results of the study by De Waele et al. (2013) including the same *O. sativa* genotypes. In both studies a large variability in susceptibility to *M. graminicola* infection of the *O. sativa* genotypes included was observed. Numerous differences in susceptibility of the same *O. sativa* genotype between the two experiments were found. For instance, in this study, the aerobic genotype CT6510-24-1-2 had the lowest final nematode population among the *O. sativa* genotypes (Mf = 32) while the aerobic genotype Way Rarem had the highest final nematode population among the *O. sativa* genotypes (Mf = 203). In the study of De Waele et al. (2013), the final J2 population per root system of CT6510-24-1-2 (35000 J2) was not similar as the experiment meanwhile Way Rarem had only the third-highest final J2 population per root system (48600 J2) among the *O. sativa* genotypes. Variation in nematode reproduction, especially in *O. sativa* genotypes which are (highly) susceptible to *M. graminicola* infection, can be expected between experiments such as observed in this study and the study of De Waele et al. (2013) due to the differences in experimental conditions (differences in inoculum level, soil type, time of sampling, water regime, etc.). Pokharel et al. (2012) reported that factors such as differences in inoculum levels and inoculation method, pot size, plant growth stage, etc. may influence *M. graminicola* reproduction. Nevertheless, in spite of the differences in experimental conditions between this study and the study of De Waele et al. (2013) comparable results were observed in the host response to *M. graminicola* infection of the resistant reference genotype (TOG5675) and the other *O. glaberrima* genotypes included. We can thus conclude that the hydroponic system we used in this study is able to identify natural resources of resistance to *M. graminicola* infection in a very practical way.

In the experiment carried out by De Waele et al. (2013), the fresh root weight of each rice genotype was higher compared with the fresh root weight of the plants in this study (on average 12.5 vs. 0.28 g, respectively) because in the former experiment the plants were harvested at maturity. As a result, a much higher number of J2 per root system was recovered from the mature plants grown under aerobic conditions in the outdoor raised beds in a sandy loam soil compared with the young plants grown in the hydroponic system (on average about 35000 vs. about 4500 J2/root system). However, the average number of J2 per root unit was much higher in the plants grown in the hydroponic system compared with plants grown under aerobic conditions and this shows that even when not many root tissues are available *M. graminicola* can build up extremely high population densities inside the roots of susceptible *O. sativa* genotypes. The highest observed pathogen pressure (i.e. J2 per root unit) in the hydroponic system (this study) was about 7000 J2 per 0.1/g roots (in the aerobic genotype Way Rarem) vs. 8300 J2/g roots (in UPLRi-5) under aerobic conditions (De Waele et al. 2013).

Differences in pathogen pressure can be caused by differences in the experimental conditions in which the roots grow, the nematodes migrate, penetrate, develop and reproduce, and the pathogen and the host plant interact. Under aerobic conditions at plant maturity, nematodes could have migrated and established feeding sites in other

roots of the same plant resulting in less nematodes per unit root whereas in the hydroponic system with rice plants in their seedling stage (thus having less roots), nematode migration, etc. may have been restricted to a limited portion of the root resulting in a higher number of J2 per root unit. Unlike in the hydroponic system, Way Rarem did not have the highest number of J2 per root unit under aerobic conditions but UPLRi-5 did. Considering that Way Rarem and UPLRi-5 had comparable root weights under aerobic conditions at maturity, the observed differences in pathogen pressure may be attributed to differences in susceptibility to *M. graminicola* between the two genotypes.

As a result of the low nematode Mf on the resistant *O. glaberrima* genotypes, the severity of root galling in this study was also low on these genotypes (root galling index ranging from 0 to 0.9). On the susceptible *O. sativa* genotypes, the root galling index ranged from 1.5 (on the aerobic genotype IR78910-23-1-3-4) to 4.1 (on UPLRi-5) averaging 2.7 for all *O. sativa* genotypes. Although another root galling rating scale was used in the study by De Waele et al. (2013) based on the percentage of roots having at least one gall (Taylor & Sasser 1978), IR78910-23-1-3-4 also had the lowest root galling index (2.1), but UPLRi-5 had not the highest root galling index among the *O. sativa* genotypes. Fernandez et al. (2013) observed that galls formed on rice plants grown under flooded conditions appear to be larger than those formed under non-flooded conditions. So it may be that gall formation of plants grown in hydroponics may not be a good indicator of gall formation under other water regimes.

Differences in severity of root galling have been reported in *M. graminicola*-resistant *O. glaberrima* and *M. graminicola*-susceptible *O. sativa* genotypes as early as 3 and 7 days after inoculation (Cabasan et al. 2012). In the study of Cabasan et al. (2012), a higher number of *M. graminicola* J2 could penetrate the roots of susceptible *O. sativa* genotypes compared with resistant *O. glaberrima* genotypes while nematode development is faster in *O. sativa* roots compared with *O. glaberrima* roots resulting in a higher number of egg-laying females and a higher number of eggs laid by these females.

To our knowledge, this is the first time that a hydroponic system was used to evaluate the host response of an agricultural crop for resistance to a *Meloidogyne* species. This study shows that the susceptibility/resistance (or at least some of the resistance) of rice genotypes to *M. graminicola* infection can be determined in an early and fast way. In the hydroponic system used in this study, rice seedlings were established in SAP substrate for 7 days and the nematode Mf was assessed at 23 days after inoculation with only 50 J2/plant. Rice plants can be easily uprooted and the severity of galling easily visually rated. J2 in the nutrient solution can be easily counted and extracted from the roots without much washing. The inclusion of a susceptible and, especially, a resistant reference genotype is essential. The hydroponic system, however, has also its limitations. One of these limitations is that re-invasion of newly formed roots by J2 is very limited. But the most important limitation is that the hydroponic system is not suitable to evaluate yield loss which is the most important factor to identify tolerance of rice genotypes to *M. graminicola* infection.

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Disclosure statement

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References

Bos L, Parlevliet JE. 1995. Concepts and terminology on plant/pest relationships: toward consensus in plant pathology and crop protection. Annu Rev Phytopathol. 33:69–102.

Cabasan MTN, Kumar A, De Waele D. 2012. Comparison of migration, penetration, development and reproduction of *Meloidogyne graminicola* on susceptible and resistant rice genotypes. Nematology. 14:405–415.

Cook R, Evans K. 1987. Resistance and tolerance. In: Brown RH, Kerry BR, editors. Principles and practice of nematode control in crops. Sydney: Academic Press; p. 179–239.

De Waele D, Das K, Zhao D, Tiwari RKS, Shrivastava DK, Vera-Cruz C, Kumar A. 2013. Host response of rice genotypes to the rice root-knot nematode (*Meloidogyne graminicola*) under aerobic soil conditions. Arch Phytopathol Plant Prot. 46:670–681.

Dochez C, Whyte J, Tenkouano A, Ortiz R, De Waele D. 2005. Response of East African highland bananas and hybrids to *Radopholus similis*. Nematology. 7:655–666.

Fernandez L, Cabasan MTN, De Waele D. 2013. Life cycle of the rice root-knot nematode *Meloidogyne graminicola* at different temperatures under non-flooded and flooded conditions. Arch Phytopathol Plant Prot. 47:1042–1049. doi:10.1080/03235408.2013.829627

Hussey H, Janssen GJW. 2002. Root-knot nematode: *Meloidogyne* species. In: Starr JL, Cook R, Bridge J, editors. Plant resistance to parasitic nematodes. Wallingford: CAB International; p. 43–70.

Ji H, Gheysen G, Denil S, Lindsey K, Topping JF, Nahar K, Haegeman A, De Vos WH, Trooskens G, Van Criekinge W, De Meyer T, Kydnt T. 2013. Transcriptional analysis through RNA sequencing of giant cells induced by *Meloidogyne graminicola* in rice roots. J Exp Bot. 64:3885–3898. doi:10.1093/jxb/ert219

Lambert KN, Tedford EC, Caswell EP, Williamson VM. 1992. A system for continuous production of root-knot nematode juveniles in hydroponic culture. Phytopathology. 82:512–515.

Oka Y, Mizukubo T. 2009. Tomato culture filtrate stimulates hatching and activity of *Meloidogyne incognita* juveniles. Nematology. 11:51–61.

Plowright RA, Coyne DL, Nash P, Jones MP. 1999. Resistance to the rice nematodes *Heterodera sacchari*, *Meloidogyne graminicola* and *M. incognita* in *Oryza glaberrima* and *O. glaberrima* x *O. sativa* interspecific hybrids. Nematology. 1:745–751.

Pokharel RR, Abawi GS, Duxbury JM. 2011. Greenhouse evaluation of rice and wheat germplasms for resistance to *Meloidogyne graminicola* with comments on evaluation indices and proposal of a new one. Nematol Medit. 39:157–168.

Pokharel RR, Duxbury JM, Abawai G. 2012. Evaluation of protocol for assessing the reaction of rice and wheat germplasm to infection by *Meloidogyne graminicola*. J Nematol. 44:274–283.

Reversat G, Boyer J, Sannier C, Pando-Bahuon A. 1999. Use of a mixture of sand and water-absorbent synthetic polymer as substrate for the xenic culturing of plant-parasitic nematodes in the laboratory. Nematology. 1:209–212.

Reversat G, Fernandez L. 2004. Effect of inoculations with single and multiple juveniles on release of progeny of *Meloidogyne graminicola* from susceptible rice. Nematology. 6:1–6.

Seinhorst JW. 1950. De betekenis van de toestand van de grond voor het optreden van aantasting door het stengelaaltje (*Ditylenchus dipsaci* (Kühn) Filipjev). Tijdschr. Plantenziekten. 56: 288–348.

Soriano IR, Schmit V, Brar DS, Prot J, Reversat G. 1999. Resistance to rice root-knot nematode *Meloidogyne graminicola* identified in *Oryza longistaminata* and *O. glaberrima*. Nematology. 1:395–398.

Starr JL, Bridge J, Cook R. 2002. Resistance to plant parasitic nematodes: history, current use future potential. In: Starr JL, Cook R, Bridge J, editors. Plant resistance to parasitic nematodes. Wallingford: CAB International; p. 1–22.

Starr JL, Mercer CF. 2009. Development of resistant varieties. In: Perry RN, Moens M, Starr JL, editors. Root-knot nematodes. Wallingford: CABI Publishing; p. 326–337.

Synder DW, Opperman CH, McK BDD. 2006. A method for generating *Meloidogyne incognita* males. J Nematol. 38:192–194.

Taylor AL, Sasser JN. 1978. Biology, identification and control of root-knot nematodes (Meloidogyne species). Raleigh, North Carolina: North Carolina State University Graphics; p. 1–111.

Villanueva LM, Prot J-C, Matias DM. 1992. Plant parasitic nematodes associated with upland rice in the Philippines. J Plant Prot Trop. 9:143–149.

Win PP, Kyi PP, Maung ZTZ, De Waele D. 2013. Evaluation of the host response of lowland and upland rice varieties from Myanmar to the rice root-knot nematode *Meloidogyne graminicola*. Arch Phytopathol Plant Prot. 47:869–891. doi:10.1080/03235408.2013.824640